

We claim:

1. An immunostimulatory composition that comprises an immunostimulating effective amount of a melanin preparation as an extract of one of the following botanicals and any
5 combination thereof: *Echinacea*, American ginseng, black walnut, green tea, Parthenium integrifolium, Korean ginseng, alfalfa sprouts, ginger, goldenseal, red clover, dandelion, black cohosh, licorice, chamomile, milk thistle, alfalfa, horsetail, astragalus, gotu kola, feverfew, valerian, hawthorn, rosemary, saw palmetto, ephedra, pau d'arco, ginkgo, garlic, St. John's wort, *Agaricus bisporus* (common mushroom), *Agaricus bisporus* brown strain (portabella
10 mushroom), *Lentinus edodes* (shiitake mushroom) or *Boletus edulis* (porcini mushroom).
2. The immunostimulatory composition of claim 1, wherein the melanin preparation comprises an extract of *Echinacea*, American ginseng, black walnut, green tea, Parthenium integrifolium, Korean ginseng, alfalfa sprouts, ginger, goldenseal, red clover, dandelion, black
15 cohosh, licorice, chamomile, milk thistle, alfalfa, horsetail, astragalus, gotu kola.
3. The immunostimulatory composition of claim 1, wherein the melanin preparation yields at least one of following degradation products, when subjected to pyrolysis-GC-MS: toluene, phenol, 4-methylphenol, indole, 7-methylindole, ethylbenzene, 3-methylpyrrole, styrene,
20 benzene acetonitrile, benzene propanenitrile.

4. The immunostimulatory composition of claim 3, wherein the melanin preparation is protein-free.

5. The immunostimulatory composition of claim 1, wherein said melanin preparation results from an aqueous phenol extract or phenol extract.

6. The immunostimulatory composition of claim 1, wherein the melanin preparation results from an extract produced by extraction with water, alcohol, weak base or any combination thereof.

7. The immunostimulatory composition of claim 1, further comprising a carrier or excipient.

8. The immunostimulatory composition of claim 1, wherein the immunostimulation is manifested by monocyte activation.

9. A method of treating a subject requiring immune mediation comprising administering to said subject an immunostimulatory cell activating amount of a melanin preparation of claim 1.

10. An immunostimulatory agent, comprising:

an immunostimulatory effective amount of a melanin preparation, wherein the melanin preparation comprises an extract from at least one of the following botanicals: *Echinacea*, American ginseng, black walnut, green tea, *Parthenium integrifolium*,

Korean ginseng, alfalfa sprouts, ginger, goldenseal, red clover, dandelion, black cohosh, licorice, chamomile, milk thistle, alfalfa, horsetail, astragalus, gotu kola, feverfew, valerian, hawthorn, rosemary, saw palmetto, ephedra, pau d'arco, ginkgo, garlic, St. John's wort, *Agaricus bisporus* (common mushroom), *Agaricus bisporus* brown strain (portabella mushroom), *Lentinus edodes* (shiitake mushroom), *Boletus edulis* (porcini mushroom).

11. The immunostimulatory agent of claim 10, wherein the melanin preparation comprises an extract of *Echinacea*, American ginseng, black walnut, green tea, *Parthenium integrifolium*, Korean ginseng, alfalfa sprouts, ginger, goldenseal, red clover, dandelion, black cohosh, licorice, chamomile, milk thistle, alfalfa, horsetail, astragalus, gotu kola.

12. The immunostimulatory agent of claim 10, wherein the melanin preparation yields at least one of the following degradation products, when subjected to pyrolysis-GC-MS: toluene, phenol, 4-methylphenol, indole, 7-methylindole, ethylbenzene, 3-methylpyrrole, styrene, benzene acetonitrile, benzene propanenitrile.

13. The immunostimulatory agent of claim 12, wherein the melanin preparation is protein-free.

14. The immunostimulatory agent of claim 10, wherein the melanin preparation results from an aqueous phenol extract or phenol extract.

15. The immunostimulatory agent of claim 10, wherein the melanin preparation results from an extract produced by extraction with water, alcohol, weak base or any combination thereof.

16. A method of activating an immune cell having a receptor that recognizes melanin in a

5 subject, comprising:

providing an effective immune cell activating amount of the composition of claim 1; and
introducing said extract to said subject.

17. The method of claim 16, wherein the immune cell is a monocyte.

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18. A method of measuring an immunostimulating effective amount of an
immunostimulating melanin, comprising the following steps:

a. providing a plant material selected from the group of *Echinacea*, American
ginseng, black walnut, green tea, *Parthenium integrifolium*, Korean ginseng, alfalfa sprouts,
15 ginger, goldenseal, red clover, dandelion, black cohosh, licorice, chamomile, milk thistle, alfalfa,
horsetail, astragalus, gotu kola, feverfew, valerian, hawthorn, rosemary, saw palmetto, ephedra,
pau d'arco, ginkgo, garlic, St. John's wort, *Agaricus bisporus* (common mushroom), *Agaricus
bisporus* brown strain (portabella mushroom), *Lentinus edodes* (shiitake mushroom), *Boletus
edulis* (porcini mushroom);

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b. extracting the plant material with a solvent;

c. collecting precipitates comprising melanin;

d. removing contaminants by a solvent wash;

- e. removing contaminants by solvent partitioning;
 - f. collecting precipitates comprising melanin;
 - g. optionally testing the collected precipitates for activation of immune cells;
 - h. optionally assigning an immunostimulatory value to said melanin preparation to
- 5 be used as a standard.

19. The method of claim 18, wherein the solvent in step (b) is at least one of phenol, aqueous phenol, alcohol, water, weak base, or any combination thereof.

10 20. The method of claim 18, wherein in step (e) the partitioning is phenol:water partitioning.

21. The method of claim 18, wherein step (e) comprises phenol/chloroform: water partitioning.

15 22. The method of claim 18, wherein step (e) removes protein contaminants to provide a protein-free melanin precipitate.

23. The method of claim 22, further comprising an amino acid test analysis step to confirm protein content.

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24. A method for employing a standard for measuring an immunostimulating effective amount of a melanin preparation, comprising:

- a. providing a standard melanin preparation with an immunostimulatory value;
 - b. providing a plant material;
 - c. extracting the plant material with a solvent;
 - d. collecting precipitates comprising melanin;
 - 5 e. removing contaminants by a solvent wash;
 - f. removing contaminants by solvent partitioning;
 - g. collecting precipitates comprising melanin;
 - h. testing the collected precipitates for activation of immune cells;
 - i. comparing the activity of the collected precipitates to the standard melanin
- 10 preparation immunostimulatory value.

25. The method of claim 24, wherein the plant material is selected from the group of *Echinacea*, American ginseng, black walnut, green tea, *Parthenium integrifolium*, Korean ginseng, alfalfa sprouts, ginger, goldenseal, red clover, dandelion, black cohosh, licorice,

15 chamomile, milk thistle, alfalfa, horsetail, astragalus, gotu kola, feverfew, valerian, hawthorn, rosemary, saw palmetto, ephedra, pau d'arco, ginkgo, garlic, St. John's wort, *Agaricus bisporus* (common mushroom), *Agaricus bisporus* brown strain (portabella mushroom), *Lentinus edodes* (shiitake mushroom), *Boletus edulis* (porcini mushroom), and any combination thereof.

20 26. The method of claim 24, wherein in step (c) the solvents are at least one of aqueous phenol, phenol, alcohol, water, weak base, or any combination thereof.

27. The method of claim 24, wherein in step (f) the partitioning is phenol:water partitioning.

28. The method of claim 24, wherein step (f) comprises phenol/chloroform: water partitioning.

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29. The method of claim 24, wherein step (f) removes protein contaminants to provide a protein-free melanin precipitate.

30. A method for preparing a product containing a standardized amount of

10 immunostimulatory melanin, comprising:

- a. providing a standard melanin preparation with an immunostimulatory value;
- b. providing a melanin product;
- c. extracting the melanin product with a solvent;
- d. collecting precipitates comprising melanin;
- 15 e. removing contaminants by a solvent wash;
- f. removing contaminants by solvent partitioning;
- g. collecting precipitates comprising melanin;
- h. testing the collected precipitates for activation of immune cells;
- i. comparing the activity of the collected precipitates to the standard melanin

20 preparation immunostimulatory value to determine a standardized activity value of the product.

31. The method of claim 30, wherein step (c) the solvents are at least one of aqueous phenol, phenol, alcohol, water, weak base or any combination thereof.

32. The method of claim 30, wherein the melanin product is whole plant material.

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33. The method of claim 32, wherein the whole plant material is plant material selected from a plant from at least one of: *Echinacea*, American ginseng, black walnut, green tea, *Parthenium integrifolium*, Korean ginseng, alfalfa sprouts, ginger, goldenseal, red clover, dandelion, black cohosh, licorice, chamomile, milk thistle, alfalfa, horsetail, astragalus, gotu kola, feverfew, valerian, hawthorn, rosemary, saw palmetto, ephedra, pau d'arco, ginkgo, garlic, St. John's wort, *Agaricus bisporus* (common mushroom), *Agaricus bisporus* brown strain (portabella mushroom), *Lentinus edodes* (shiitake mushroom), *Boletus edulis* (porcini mushroom).

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34. The method of claim 33, wherein the melanin product is a melanin extract preparation.

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35. The method of claim 34, wherein the melanin extract preparation is an extract of one of the following botanicals and any combination thereof: *Echinacea*, American ginseng, black walnut, green tea, *Parthenium integrifolium*, Korean ginseng, alfalfa sprouts, ginger, goldenseal, red clover, dandelion, black cohosh, licorice, chamomile, milk thistle, alfalfa, horsetail, astragalus, gotu kola, feverfew, valerian, hawthorn, rosemary, saw palmetto, ephedra, pau d'arco, ginkgo, garlic, St. John's wort, *Agaricus bisporus* (common mushroom), *Agaricus bisporus*

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brown strain (portabella mushroom), *Lentinus edodes* (shiitake mushroom) or *Boletus edulis* (porcini mushroom).

36. A method of treating, preventing, or ameliorating a condition or disease in a subject
5 requiring enhanced immune system support; comprising providing an effective immune cell
activating amount of the composition of claim 1, and introducing said composition to said
subject.

37. The method of claim 36, wherein the condition or disease is an immune deficiency.

38. The method of claim 36, wherein the condition or disease is cancer.

39. The method of claim 36, wherein the condition or disease is a fungal, viral, or bacterial
infection.

40. The method of claim 36, wherein the condition or disease is a wound.

41. A method of preparing an extract enriched for immunostimulatory melanin, comprising:

- a. extraction of plant material with a solvent;
- b. enriching for immunostimulatory melanin by precipitation and collection of melanin;
- c. optionally washing precipitated melanin with a solvent to remove contaminants.

42. The method of claim 41, wherein step (a) comprises a water solvent, alcohol solvent, or a mixture thereof.

43. The method of claim 41, wherein the solvent in step (a) comprises a weak base.

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44. The method of claim 41, wherein the solvent in step (a) comprises at least one of a weak base, an alcohol, and mixtures thereof.

45. The method of claim 41, wherein the solvent in step (a) comprises ammonium hydroxide.

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46. The method of claim 41, wherein step (b) comprises precipitation of melanin preparation by addition of acid.

47. The method of claim 41, wherein the solvent in step (c) comprises alcohol.

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48. A melanin preparation obtained by extracting a plant or edible fungi with a solvent; wherein the melanin preparation is immunostimulatory in vitro and in vivo, and upon pyrolysis-GC-MS yields at least one of the following: toluene, phenol, 4-methylphenol, indole, 7-methylindole, ethylbenzene, 3-methylpyrrole, styrene, benzene acetonitrile, benzene propanenitrile.

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49. A method for enhancing the activity of a melanin within a plant, comprising:

(1) providing a plant;

(2) treating said plant with an elicitor of secondary metabolite production.

50. The method of claim 49, further comprising extracting melanin from elicitor treated
5 plant.

51. The method of claim 49, wherein the elicitor is chitin.

52. The method of claim 49, wherein the elicitor comprises at least one the following: chitin,
10 salicylic acid, methyl jasmonate, glucan, UV light, beta-amino butyric acid, or physical damage
(wounding).